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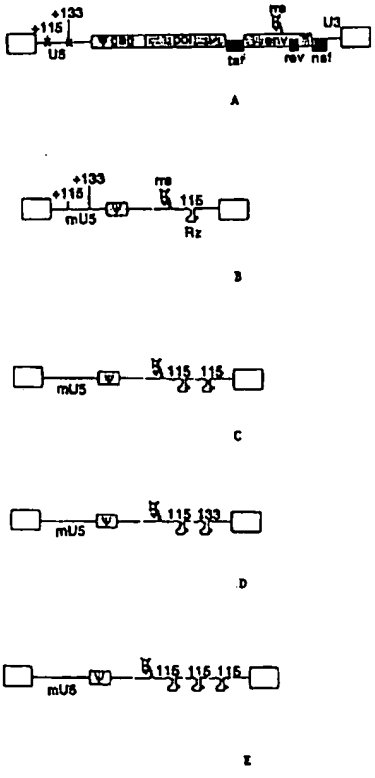
(54) Title: CONDITIONALLY REPLICATING VIRAL VECTORS AND THEIR USE

(57) Abstract

The present invention provides a conditionally replicating viral vector, methods of making, modifying, propagating and selectively packaging, and using such a vector, isolated molecules of specified nucleotide and amino acid sequences relevant to such vectors, a pharmaceutical composition and a host cell comprising such a vector, the use of such a host cell to screen drugs. The methods include the prophylactic and therapeutic treatment of viral infection, in particular HIV infection, and, thus, are also directed to viral vaccines and the treatment of cancer, in particular cancer of viral etiology. Other methods include the use of such conditionally replicating viral vectors in gene therapy and other applications.



36. A method of propagating and selectively packaging a conditionally replicating vector without using a packaging cell line, which method comprises:

(a) contacting the conditionally replicating vector with a cell capable of being infected by another vector, which is the same type of vector as the conditionally replicating vector and which differs from the conditionally replicating vector by being wild-type for replication competency;

(b) contacting the cell of (a) with said another vector of (a); and

(c) culturing the cell of (b) under conditions conducive to the propagation of said conditionally replicating vector.

37. An isolated and purified nucleic acid molecule selected from the group consisting of a DNA molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 6, 7, 15, 16, 17 and 18 and a RNA molecule comprising a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 6, 7, 15, 16, 17 and 18.

38. A method of inhibiting the replication of a wild-type strain of a virus in a host cell, which method comprises contacting the host cell, which is capable of being infected with the wild-type strain of the virus, with a vector of claim 1, the presence, transcription or translation of which inhibits the replication of the wild-type strain of virus in the host cell.

39. A method of inhibiting the replication of a wild-type strain of a virus in a host cell, which method comprises contacting the host cell, which is capable of being infected with the wild-type strain of the virus, with a vector of claim 14, the presence, transcription or

WHAT IS CLAIMED IS:

1. A conditionally replicating viral vector, which is characterized by a capacity to replicate only in a host cell that is permissive for replication of said vector, and wherein said vector comprises at least one nucleic acid sequence, the presence, transcription or translation of which confers to said vector in a host cell, which is permissive for replication of said vector, a selective advantage over a wild-type strain of virus corresponding to the virus from which said vector was derived or a helper.

2. The conditionally replicating viral vector of claim 1, wherein said at least one nucleic acid sequence comprises a nucleotide sequence, which comprises or encodes, in which case it expresses, a genetic antiviral agent, which adversely affects the replication and/or expression of a virus other than said vector.

3. The conditionally replicating viral vector of claim 2, wherein said genetic antiviral agent is selected from the group consisting of an antisense molecule, a ribozyme, and an immunogen.

4. The conditionally replicating viral vector of claim 2, wherein said genetic antiviral agent is a ribozyme.

5. The conditionally replicating viral vector of claim 4, wherein said vector is derived from a human immunodeficiency virus.

6. The conditionally replicating viral vector of claim 4, wherein said vector is derived from a Togaviridae.

7. The conditionally replicating viral vector of claim 4, wherein the catalytic domain of said ribozyme cleaves the nucleotide sequence of SEQ ID NO: 3.

8. The conditionally replicating viral vector of claim 4, wherein said ribozyme is encoded by a sequence selected from the group consisting of SEQ ID NO: 4 and SEQ ID NO: 5.

9. The conditionally replicating viral vector of claim 5, wherein said wild-type strain of virus comprises a nucleotide sequence encoded by SEQ ID NO: 1 and said vector, if DNA, comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18, and said vector, if RNA, comprises a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18.

10. The conditionally replicating viral vector of claim 5, wherein said vector lacks the *tat* gene and its splice site from the genome of the human immunodeficiency virus, wherein said human immunodeficiency virus is wild-type.

11. The conditionally replicating viral vector of claim 10, wherein, in place of said *tat* gene and its splice site, said vector comprises a triple anti-Tat ribozyme cassette, wherein the catalytic domain of each ribozyme of the triple ribozyme cassette cleaves a different site on a wild-type human immunodeficiency viral nucleic acid molecule.

12. The conditionally replicating viral vector of claim 11, wherein said wild-type human immunodeficiency viral nucleic acid molecule comprises *tat* and the

catalytic domain of each ribozyme of the triple ribozyme cassette cleaves a different site within *tat*.

13. The conditionally replicating viral vector of claim 11, wherein the catalytic domain of each ribozyme cleaves a nucleotide sequence in a region of a nucleic acid molecule of wild-type human immunodeficiency virus for which there is no ribozyme-resistant counterpart in the vector, itself.

14. A conditionally replicating viral vector, which is characterized by a capacity to replicate only in a host cell that is permissive for replication of said vector, and wherein said vector comprises at least one nucleic acid sequence, the presence, transcription or translation of which confers to a host cell, which is infected with said vector, a selective advantage over a cell infected with a wild-type strain of virus corresponding to the virus from which said vector was derived or a helper.

15. The conditionally replicating viral vector of claim 14, wherein said at least one nucleic acid sequence comprises a nucleotide sequence encoding a multidrug resistance.

16. The conditionally replicating viral vector of claim 15, wherein said vector is derived from a human immunodeficiency virus.

17. The conditionally replicating viral vector of claim 15, wherein said vector is derived from a Togaviridae.

18. The conditionally replicating viral vector of claim 16, wherein said at least one nucleic acid sequence comprises a nucleotide sequence selected from the group

consisting of a nucleotide sequence encoding a mutant protease and a nucleotide sequence encoding a mutant reverse transcriptase.

19. The conditionally replicating viral vector of claim 1, wherein said vector further comprises at least one additional nucleic acid sequence, the presence, transcription or translation of which confers to a host cell, which is infected with said vector, a selective advantage over a cell infected with a wild-type strain of virus corresponding to the virus from which said vector was derived or a helper.

20. The conditionally replicating viral vector of claim 19, wherein said at least one additional nucleic acid sequence comprises a sequence encoding a multidrug resistance.

21. The conditionally replicating viral vector of claim 19, wherein said vector is derived from a human immunodeficiency virus.

22. The conditionally replicating viral vector of claim 19, wherein said vector is derived from a Togaviridae.

23. The conditionally replicating viral vector of claim 21, wherein said at least one additional nucleic acid sequence comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence encoding a mutant protease and a nucleotide sequence encoding a mutant reverse transcriptase.

24. The conditionally replicating viral vector of claim 23, wherein said vector is selected from the group consisting of crHIV-1.1, crHIV-1.11, crHIV-1.12, and crHIV-1.111.

25. A pharmaceutical composition comprising a vector of claim 1 and a pharmaceutically acceptable carrier.

26. A pharmaceutical composition comprising a vector of claim 14 and a pharmaceutically acceptable carrier.

27. A pharmaceutical composition comprising a vector of claim 19 and a pharmaceutically acceptable carrier.

28. A host cell comprising a vector of claim 1.

29. A host cell comprising a vector of claim 14.

30. A host cell comprising a vector of claim 19.

31. A vector, wherein said vector, if DNA, comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18 and wherein said vector, if RNA, comprises a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18.

32. A method of engendering a vector, which is derived from a wild-type human immunodeficiency virus and which is capable of replicating only in a host cell that is permissive for replication of said vector, with a ribozyme, which is comprised within or encoded by said vector and which cleaves a nucleic acid of a wild-type human immunodeficiency virus but not the vector, itself, and its transcripts, if any, which method comprises:

(a) obtaining a vector, which is derived from a wild-type human immunodeficiency virus and which is

capable of replicating only in a host cell that is permissive for replication of said vector; and

(b) incorporating into the vector of (a) a nucleic acid sequence, which comprises or encodes, in which case it also expresses, a ribozyme, the catalytic domain of which cleaves a nucleic acid of a wild-type human immunodeficiency virus but not the vector, itself, and its transcripts, if any.

33. The method of claim 32, wherein step (b) comprises:

(i) deleting from said vector a nucleotide sequence comprising or encoding the U5 sequence of the wild-type human immunodeficiency virus; and

(ii) inserting into the vector of (i) a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 6, 7, 15, 16, 17 and 18, if the vector is DNA, and a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 6, 7, 15, 16, 17 and 18, if the vector is RNA.

34. The method of claim 32, wherein said vector replicates in a host cell permissive for replication of said vector more than once.

35. A method of modifying a vector, which method comprises:

(a) obtaining a vector; and

(b) introducing into the vector of (a) a nucleotide sequence selected from the group consisting of the DNA sequences of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18, if the vector is DNA, and a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18, if the vector is RNA.